[illegible]

[illegible]

Other supp[illegible]

[illegible]

EFFECT [illegible] YOUTH ON THE SEVERITY OF ISCHAEMIC INJURY IN RABBIT [illegible] SUBJECTED TO [illegible] LIGATION

[illegible]

Edlund A. S[illegible] [illegible]

The [illegible] and Cellular Cardi[illegible] 18 [illegible]

Other support: Swedish Society of Med[illegible] and the Swe[illegible] Medical Research Council.

From the De[illegible] Physiol[illegible] Karolinska [illegible] University [illegible]

1002[illegible]

Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

IV. *Neuropharmacology and Physiology*

NICOTINE ALTERS CATECHOLAMINES AND ELECTROCORTICAL ACTIVITY IN PERFUSED MOUSE BRAIN

Nicotine differentially altered electrocortical (ECoG) activity and brain catecholamine metabolism in mice (C3H and C57BL) known to differ in behavioral response to nicotine. Nicotine appeared to produce a concentration dependent desynchronization to ECoG activity in isolated perfused mouse brain (IPMB) from C3H mice. Homovanillic acid (HVA) production was unchanged in C3H perfused brains while an apparent reduction in 3-methoxy-4-hydroxyphenethyleneglycol (MPHG) was observed. Brain content of norepinephrine and dopamine remained relatively constant in the various regions tested. In IPMB from C57BL mice, nicotine elicited an enhancement of ECoG amplitude which was accompanied by decreased HVA production rates. A downward trend in MHPG production was also observed. These effects were associated with increased levels of norepinephrine and dopamine in various brain regions.

Erwin, V. G., Cornell, K. and Towell, J. F.

Pharmacology, Biochemistry & Behavior **24**(1):99-105, 1986

Other support: National Institute of Drug Abuse

From the School of Pharmacy, University of Colorado, Boulder

ACUTE CONTINUOUS EXPOSURE TO CIGARETTE SMOKE PRODUCES DISCRETE CHANGES IN CHOLECYSTOKININ AND SUBSTANCE P LEVELS IN THE HYPOTHALAMUS AND PREOPTIC AREA OF THE MALE RAT

By means of a Walton Horizontal Smoking Machine, male rats were exposed to the smoke from [illegible] cigarettes burned in a continuous fashion. Cholecystokinin (CCK) and substance P levels (determined by means of radioimmunoassay) were measured in [illegible] and preoptic regions. Acute continuous exposure to cigarette smoke [illegible] in CCK levels in the paraventricular hypothalamic region as well as [illegible] the median eminence. Furthermore, this treatment resulted in decreased CCK and substance P levels in the medial preoptic region. The results have been interpreted to indicate that CCK and substance P containing neuronal [illegible] by cholinergic nicotine-like receptors.

Pure, K. [illegible]

Acta Physiologica Scandinavica **125**:437-443, 1985

Other support: Svenska Tobaks AB, Stockholm, Sweden

From the Department of Histology, Karolinska Institute, Stockholm, Sweden; Re[illegible] Department Laboratory, Department of Obstetrics and Gynecology, [illegible] Republic of [illegible]

Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

EFFECTS OF INDOMETHACIN ON DOPAMINE-, HYDROXYETHYLTHEOPHYLLINE- AND ISOPROTERENOL-STIMULATED RENAL BLOOD FLOW IN THE CONSCIOUS DOG

[illegible]

[illegible]

[illegible]

CLONIDINE TREATMENT ELEVATES CONTENT OF NEUROPEPTIDE Y IN CARDIAC NERVES

[illegible] clonidine lowers blood pressure by means of central inhibition of sympathetic nerve activity. Recently, neuropeptide Y (NPY) has been shown to coexist with noradrenaline (NA) in, for example, cardiovascular nerves and to [illegible] an antihypertensive drug receptor has been shown to cause depletion [illegible] of NA but also of NPY. The main peculiarity of NPY-LI is its tissue [illegible] due to increased sympathetic nerve activity. Here we report that these two antihypertensive drugs, both known to impair sympathetic noradrenergic transmission via different modes of action, selectively affect the NPY [illegible] in opposite directions. Thus, in contrast to the reduction seen after [illegible] treatment, the clonidine regimen actually increased the cardiac levels of NPY-LI which is located in sympathetic nerves.

The consequence of both reserpine and clonidine treatment ultimately would be an impairment of NPY function in the cardiac sympathetic nerves, either due to depletion or reduced release. Since NPY exerts potent vasoconstrictor effects, the present findings and considerations may have bearing on the antihypertensive actions of the drugs as well as rebound phenomena after chronic withdrawal.

[illegible] *Acta Physiologica Scandinavica* [illegible] *Life Sciences* [illegible]

[illegible] 128, 1985.

Other support: The Swedish Medical Research Council, the Swedish Tobacco Company, Petrus and Augusta Hedlunds Stiftelse, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institutet, Stockholm, and Swedish

CAPSAICIN-SENSITIVE NERVES AND URETERIC MOTILITY: OPPOSING EFFECTS OF TACHYKININS AND CALCITONIN GENE-RELATED PEPTIDE

A dense network of capsaicin-sensitive sensory nerves is present in the guinea pig ureter. These peripheral branches of sensory neurons contain several peptides including multiple tachykinins (substance P (SP), neurokinin A (NKA), neuropeptide K (NKK) and calcitonin gene-related peptide (CGRP). In the present study we have investigated the possible influence of capsaicin-sensitive sensory nerves and of the two peptides on ureteric motility *in vivo*.

Capsaicin caused inhibition of the ureteric contractions at 0.1 mg/kg, which the highest capsaicin dose (100 mg/kg) induced an initial inhibition, which was followed by a long-lasting stimulation of motility. The capsaicin effects were absent in capsaicin-pretreated animals. The CGRP inhibited ureteric contractions, while NKA caused a long-lasting stimulation of motility. The CGRP and NKA effects were still present in [illegible] treated animals. Electrical stimulation of the ureteric nerves in the ganglion [illegible] inhibition of ureteric motility, which was followed by a long-lasting [illegible]. This nerve stimulation evoked responses were also present [illegible] with capsaicin, the latency and amplitude of the [illegible]

[illegible]

Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

Other support: Austrian Scientific Research Funds, the Swedish Medical Research Council, the Swedish Tobacco Company and the Swiss National Science Foundation.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CAPSAICIN INDUCED RELEASE OF MULTIPLE TACHYKININS (SUBSTANCE P, NEUROKININ A AND ELEDOISIN-LIKE MATERIAL) FROM GUINEA PIG SPINAL CORD AND URETER

The release of tachykinins from isolated slice preparations of the guinea-pig spinal cord and ureter was studied *in vitro*. Capsaicin (10 μM) caused release of substance P, neurokinin A and an eledoisin-like component from both the spinal cord and ureter. The release of tachykinins induced by capsaicin or potassium (60 mM) was calcium dependent. No detectable release of neurokinin B or neuropeptide K, an N-terminally extended form of neurokinin A, was induced by capsaicin. No detectable release of tachykinins could be demonstrated after exposure to agents which are known to activate C-fiber afferents, such as histamine, bradykinin, serotonin, prostaglandins E_1, E_2 or acetylcholine. Protein extravasation in the ureter, as determined by the Evans Blue extravasation technique was used as a functional correlate to the tachykinin release. Protein extravasation was induced *in vivo* by local intraluminal injections of capsaicin at several hundred-fold lower concentrations than those required to induce a detectable release of tachykinins *in vitro*. The difference may, however, partly depend on the experimental conditions and the detection limit of the tachykinin assay used. The protein extravasation response to capsaicin was absent after systemic capsaicin pretreatment, which causes a marked depletion of tachykinins in the ureter. In conclusion, capsaicin evokes release of several tachykinins from both central and peripheral endings of primary afferent neurons. The peptides released from sensory nerves in the periphery may induce effects such as protein extravasation and smooth muscle contraction.

Hua, X.-Y., Saria, A., Gamse, R., and *Lundberg, J. M.*

Neuroscience **19**(1):313-319, 1986.

Other support: Swedish Medical Research Council, Austrian Scientific Research Funds, the Swedish Tobacco Company, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

SPECIFIC RECEPTOR AND CARDIOVASCULAR EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE

Specific binding sites for calcitonin gene-related peptide (CGRP) were demonstrated in the rat heart and spleen. Autoradiography revealed rat [^{125}I]iodo-CGRP binding associated with the intima and media of the aorta, the coronary arteries and the heart valves, and the red pulp of the spleen. Half-maximal inhibition of rat [^{125}I]iodo-CGRP binding to membranes of the rat atria and the spleen was obtained with, respectively, 5 and 0.35 nM unlabeled rat CGRP; these values correspond to EC_x values of 3 and 0.14 nM for activation of adenylate cyclase by CGRP. In the isolated, spontaneously beating right atrium, the EC_x values of stimulation of the force and rate of contraction by rat CGRP were 120 and 70 nM, respectively. Rat CGRP caused relaxa-

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

tion of splenic strips, precontracted with noradrenaline; the EC_x was 50 nM. The β-adrenergic blocking agent metoprolol, while obliterating the increase in the force and rate of contraction evoked by noradrenaline in the right atrium, did not significantly change the action of CGRP. Similarly, preserved action of CGRP in the presence of indomethacin as well as mepyramine and cimetidine argues against a role of prostaglandins or histamine in the functional responses of CGRP. Much like CGRP, capsaicin, which releases mediators from sensory neurons, caused stimulation of the force and rate of contraction of the isolated right rat atrium. After tachyphylaxis to CGRP, the response to noradrenaline was intact, while the positive chronotropic and inotropic effects of capsaicin were suppressed. The results indicate that the cardiac effects of capsaicin may be due to the release of endogeneous CGRP through a local mode of action.

Sigrist, S., Franco-Cereceda, A., Muff, R., and *Lundberg, J. M.*

Endocrinology **119**(1):381-389, 1986.

Other support: Swiss National Science Foundation, the Swedish Medical Research Council, the Swedish Tobacco Company, the Hedlunds Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

DUAL CAPSAICIN EFFECTS ON URETERIC MOTILITY: LOW DOSE INHIBITION MEDIATED BY CALCITONIN GENE-RELATED PEPTIDE AND HIGH DOSE STIMULATION BY TACHYKININS?

The effects of capsaicin, in relation to substance P (SP), neurokinin A (NKA), neuropeptide K (NPK) and calcitonin gene-related peptide (CGRP) which coexist in local sensory nerves, on the motility of the guinea-pig ureter were studies *in vivo* and *in vitro*. Capsaicin in a *low dose* (10 nmol kg^{-1}) given i.v. inhibited spontaneous, peristaltic contractions, as revealed by perfusion-pressure changes of the constantly perfused ureter *in vivo*. This action was independent of autonomic reflexes and prostaglandin formation. Capsaicin stimulated ureteric motility at *higher doses* (100 and 500 nmol kg^{-1}). The dual effects of capsaicin on the ureteric contractility were absent 2 weeks after systemic capsaicin treatment, which depletes sensory neuropeptides. Both NKA and NPK initiated, as well as increased, the magnitude of the peristaltic contractions of the ureter, while SP only caused a minor excitatory effect. The CGRP inhibited spontaneous, as well as NKA- and NPK-induced ureteric peristaltic contractions. *In vitro* experiments on the ureter revealed that capsaicin (10^{-8} M) induced phasic circular muscle contractions in 60% of the experiments. Neurokinin A, NPK and SP consistently increased the contractile activity. The NKA tachyphylaxis inhibited the contractile response to other tachykinins and capsaicin. The SP analogue Spantide (/D-Arg^1, D-$Trp^{7,9}$, Leu^{11}/-SP) inhibited the contractile responses to SP, NKA and NPK. The CGRP also inhibited the NKA- and NPK-induced contractions of the ureter *in vitro*. In conclusion, capsaicin, which induces the release of mediators from sensory nerves within the ureter, has either stimulatory or inhibitory effects on ureteric smooth muscle, depending on the *in vivo* dose administered. The inhibitory response at a low capsaicin dose is similar to the effect of CGRP, while the contractile effects at higher doses resemble the response to tachykinins.

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

Hua, X.-Y. and *Lundberg, J. M.*

Acta Physiologica Scandinavica **128**:453-465, 1986.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Swedish Work Environment Fund, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CO-RELEASE OF NEUROPEPTIDE Y AND CATECHOLAMINES UPON ADRENAL ACTIVATION IN THE CAT

The release of neruopeptide Y (NPY)-like immunoreactivity (-LI) in relation to catecholamines from the cat adrenal was studied in anaesthetized animals. Abdominal surgery increased plasma levels of NPY-LI from 65 ± 6 to 149 ± 26 pmol l^{-1}. A positive veno–arterial concentration gradient over the adrenal gland was found for both NPY-LI, adrenaline (Adr) and noradrenaline (NA) during basal conditions. Asphyxia for 2 min increased the output of both NPY-LI and catecholamines from the adrenal. Electrical stimulation of the splanchnic nerve caused a marked increase in adrenal output of NPY-LI and catecholamines. The adrenal content of NPY-LI, as well as the release of NPY-LI from the adrenal, was at least 1000-fold lower on a molar basis than that of catecholamines. The concentration of NPY-LI in the adrenal vein upon splanchnic nerve stimulation was in the nM range. Reversed-phase HPLC characterization revealed that NPY-LI in the adrenal, and in the adrenal venous plasma collected during splanchnic nerve stimulation, was closely related to synthetic porcine NPY. Stimulation with bursts of 20 Hz for 1 s with 10 s intervals for 2 min caused a four-fold higher output of NPY-LI and Adr compared to a continuous stimulation with 2 Hz, giving the same number of impulses. The NA output, however, was only slightly increased by burst stimulation. Guanethidine did not reduce the adrenal output of NPY-LI or catecholamines induced by splanchnic nerve stimulation, while the release was abolished by chlorisondamine. NPY enhanced the *in vitro* contractile activity of Adr and NA on the a. profunda femoris from the cats used in the functional experiments. The threshold concentration of NPY for this enhancement was 250 pM. The NPY in higher concentrations (> 10^{-8} M) had contractile effects *per se*. In conclusion, the present data suggest a functional relationship between adrenal release and effects of NPY and Adr.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica **126**:231-238, 1986.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CO-RELEASE OF NEUROPEPTIDE Y AND CATECHOLAMINES DURING PHYSICAL EXERCISE IN MAN

Venous plasma levels of neuropeptide Y-like immunoreactivity (with chromatographic properties of synthetic neuropeptide Y) increased in parallel with catecholamines, heart rate and blood pressure during graded physical exercise in man. The plasma levels of neuropeptide Y correlated better with the levels of noradrenaline than

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

adrenaline, suggesting release of a neural origin. Taken together with previous results, this suggests that neuropeptide Y is released together with noradrenaline upon sympathetic activation during physiological conditions in man. Determinations of plasma neuropeptide Y may therefore be valuable in the assessment of sympathetic nerve activity.

Lundberg, J. M., et al.

Biochemical and Biophysical Research Communications **133**(1):30-36, November 27, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Karolinska Institute, the Swedish National Association Against Heart and Chest Diseases, and the Research Council of the Swedish Sports Federation.

From the Departments of Pharmacology, Karolinska Institute, Stockholm, Sweden.

MECHANISMS UNDERLYING PRE- AND POSTJUNCTIONAL EFFECTS OF NEUROPEPTIDE Y IN SYMPATHETIC VASCULAR CONTROL

The effects of porcine neuropeptide Y (NPY) regarding sympathetic vascular control were studied *in vitro* on isolated rat blood vessels. The 10^{-8} M NPY enhanced (about two-fold) the contractile responses to transmural nerve stimulation (TNS), noradrenaline (NA) and adrenaline (about two-fold) in the femoral artery. Higher concentrations of NPY (> 10^{-8} M) caused an adrenoceptor-resistant contraction *per se*. The TNS-evoked [^{3}H]NA efflux was significantly reduced by NPY in a concentration-dependent manner (threshold 10^{-9} M). The calcium antagonist, nifedipine, abolished the contractile effects of NPY and the NPY-induced enhancement of NA contractions but did not influence the prejunctional inhibition of [^{3}H]NA release. Receptor-binding studies showed that the ratio of alpha$_1$- to alpha$_2$-adrenoceptors in the femoral artery was 30:1. The NPY did not cause any detectable change in the number of alpha$_1$- or alpha$_2$-adrenoceptor binding sites or in the affinity of alpha$_2$-binding sites, as revealed by prazosin- and clonidine-binding, respectively. The NPY also inhibited the TNS-evoked [^{3}H]NA release (by 42-86%) in the superior mesenteric and basilar arteries and in femoral and portal veins. The NPY still depresed TNS-evoked [^{3}H]NA secretion from the portal vein in the presence of phentolamine. The NPY caused a clear-cut contraction in the basilar artery, increased the contractile force of spontaneous contractions in the portal vein, while only weak responses were observed in the superior mesenteric artery and femoral vein. The NA-induced contraction was markedly enhanced by NPY in the superior mesenteric artery, only slightly enhanced in the portal vein and uninfluenced in the femoral vein. In conclusion, in all blood vessels tested, NPY depresses the TNS-evoked [^{3}H]NA secretion via a nifedipine-resistant action. Furthermore, NPY exerts a variable, CA^{2+}-dependent vasoconstrictor effect and enhancement of NA and TNS contractions.

Pernow, J., Saria, A. and *Lundberg, J. M.*

Acta Physiologica Scandinavica **126**:239-249, 1986.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Karolinska Institute and the Austrian Scientific Research Fund.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

EFFECTS OF VIP, PHM AND SUBSTANCE P ON BLOOD VESSELS AND SECRETORY ELEMENTS OF THE HUMAN SUBMANDIBULAR GLAND

The effects of the neuropeptides VIP, PHM and substance P (SP) on vascular smooth muscle tone, K^+ secretion from exocrine elements and tissue content of cyclic AMP (cAMP) in the human submandibular gland were studied *in vitro*.

All three peptides caused relaxation of nonradrenaline contracted human submandibular arteries at nM concentrations. SP was slightly more active than VIP and PHM which had a similar potency as vasodilators. Only carbachol but not VIP, PHM or Sp stimulated K^+ secretion from exocrine elements of the human submandibular gland. Principally similar *in vitro* effects on K^+ secretion were obtained on the cat submandibular gland but in the rat not only carbachol but also SP stimulated K^+ secretion. VIP and PHM increased cAMP production of exocrine elements in the human submandibular gland in nM concentrations. VIP was about 5-fold more potent than PHM with regards to cAMP production.

In conclusion, VIP, PHM and SP relaxed human submandibular arteries in vitro. Both VIP and PHM stimulated cAMP production in glandular tissue but none of the three peptides induced K^+ secretion from human submandibular gland tissue. This suggests that, in contrast to the situation in the rat, SP does not cause watery salivation in man, while VIP and PHM may modulate protein e.g., amylase content of the saliva.

Larsson, O., Duner-Engstrom, M., *Lundberg, J. M.*, and Fredholm, B. B.

Regulatory Peptides **13**:319-326, 1986.

Other support: Swedish Medical Research Council and the Swedish Tobacco Company.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NEUROPEPTIDE Y-A COTRANSMITTER WITH NORADRENALINE AND ADENOSINE 5'-TRIPHOSPHATE IN THE SYMPATHETIC NERVES OF THE MOUSE VAS DEFERENS? A BIOCHEMICAL, PHYSIOLOGICAL AND ELECTROPHARMACOLOGICAL STUDY

A combination of biochemical, physiological and electropharmacological methods was employed to examine the occurrence of neuropeptide Y and the pre- and postjunctional effects of this peptide on sympathetic neuromuscular transmission in the mouse vas deferens. This tissue had a high content of neuropeptide Y-[illegible]-immunoreactive material, suggesting a dense innervation by neuropeptide Y-containing nerve fibres. Addition of neuropeptide Y at concentrations from 5×10^{-10} to 5×10^{-7} M induced both pre- and postjunctional effects *in vitro*. Neuropeptide Y *per se* induced a rise in the resting tension, and "instantly" potentiated the contractile effects of exogenous noradrenaline and of the stable adenosine 5'-triphosphate (ATP) analogue, α,β-methylene ATP. Neuropeptide Y reduced the secretion of [^{3}H]noradrenaline evoked by electrical nerve stimulation, and selectively depressed the stimulus-evoked, but not the spontaneously occurring excitatory junction potentials in smooth muscle cells. Further, neuropeptide Y reduced the amplitudes of the twitch contractions evoked by electrical field stimulation with short stimulus trains at 10 Hz, and also (although to a smaller extent) the delayed contractile response to longer trains of nerve stimuli. The

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pre- and postjunctional effects of neuropeptide Y were not changed by α- or β-adrenoceptor blocking agents, or by tachyphylaxis to the effects of ATP, or by the calcium-channel blocker nifedipine.

In conclusion, sympathetic neuromuscular transmission in the mouse vas deferens may be mediated not only by noradrenaline and ATP, but also by neuropeptide Y. This peptide may play a dual role: initially to locally potentiate the contractile response to noradrenaline and ATP, and subsequently to locally "turn off" the secretion of transmitter in previously (hyper-?)active regions of the nerve terminals.

Stjärne, L., *Lundberg, J. M.*, Åstrand, P.

Neuroscience **18**(1):151-166, 1986.

Other support: Swedish Medical Research Council and the Swedish Tobacco Company.

From the Departments of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden.

IS SYNAPTIC TRANSMISSION MODULATED BY PROGESTERONE?

The influence of the gonadosteroid hormone progesterone on synaptic transmission was studied using the frog neuromuscular preparation. Intracellular recording of synaptic potentials revealed enhanced release of acetylcholine from motor nerve terminals exposed to progesterone (3nM-3mM). The following effects were observed. (1) An augmented quantal content of evoked release of transmitter; (2) an elevation in synaptic facilitation; and (3) a substantial increase in the rate of spontaneously occurring miniature endplate potentials. It is suggested that synaptic transmission at the neuromuscular junction may be naturally modulated by the physiologically oscillating level of progesterone.

Meiri, H. (*Rahamimoff, R.*)

Brain Research **385**:193-196, 1986.

Other support: Muscular Dystrophy Association and the U.S.-Israel Binational Science Foundation.

From the Laboratory of Cell Biology, Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

EFFECT OF *L*-METHIONINE ON CONTRACTILE RESPONSE, CALCIUM INFLUX AND CALCIUM CHANNEL BLOCKING AGENTS IN THE RAT AORTA

L-Methionine incubated with aorta strips and S-adenosyl-*L*-methionine incubated with aorta membranes methylate membrane phospholipids. *L*-Methionine enhances the contractile response of helical strips of rat aorta to KCl. *L*-Methionine also enhances the slow component of the contractile response of rat aorta to norepinephrine associated with influx of exogenous calcium. *L*-Homocysteinethiolactone inhibits

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methylation of membrane phospholipids and depresses the contractile response to KCl and to norepinephrine. *L*-Methionine enhances and *L*-homocysteinethiolactone depresses KCl-stimulated influx of calcium into rat aorta strips. *L*-Methionine has no effect on calcium efflux. Tested against calcium channel blocking agents, *L*-methionine reduces the inhibition caused by diltiazem and chlorpromazine but not that caused by TMB 8 or verapamil. It is postulated that methylated intermediates of phospholipd methylation enhance the function of membrane calcium channels.

Landon, E. J., Owens, L., and *Sastry, B. V. R.*

Pharmacology **32**:190-201, 1986.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REGULATION OF ACETYLCHOLINE RELEASE FROM RODENT CEREBRUM BY PRESYNAPTIC RECEPTORS, METHIONINE ENKEPHALIN AND SUBSTANCE P

The findings of this study indicate the operation of a homeostatic relationship between the release of ACh, MEK and SP. This relationship may be of physiological importance in the regulation of the release of ACh. If the amount of ACh in the synaptic gap is low, a positive feedback loop is triggered, causing the release of SP either directly or by a disinhibition phenomenon. Low ACh does not stimulate the negative feedback loop. Hence, no MEK is released, leading to a relief of an inhibitory action of MEK on the release of SP. The released SP would increase further release of ACh by increasing the uptake of Ca^{++}. This process would continue until a peak release of ACh was reached. At this point, the high amount of ACh in the synaptic gap would trigger a negative feedback loop, inducing the release of MEK, which in turn would limit any further release of ACh and SP by decreasing the uptake of Ca^{++}. These feedback systems seem to operate through two different types of muscarinic receptors.

Sastry, B. V. R.

Advances in Behavioral Biology **30**:1047-1056, 1986.

Other support: National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REVIEW: CHOLINERGIC SYSTEMS AND MULTIPLE CHOLINERGIC RECEPTORS IN OCULAR TISSUES

Acetylcholine (ACh), choline acetyltransferases and cholinesterases occur in cornea, iris-ciliary body complex and retina of several vertebrates. In cornea, ACh may serve as a sensory transmitter as well as a local hormone, the function of which is

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

not delineated. The function of ACh as the parasympathetic neurotransmitter at the iris and ciliary body is well established. The muscarinic receptors on the iris smooth muscle are similar to the muscarinic receptors (M2 type in two way classification) at other smooth muscles towards their interaction with agonists and antagonists. Binding studies using radiolabeled antagonists and their displacement by agonists indicate that muscarinic receptors in membranes of iris-ciliary body complex are heterogeneous indicating more than one subtype of muscarinic receptors. A subtype other than M2 receptors may occur at the presynaptic sites of parasympathetic nerves, which have yet to be investigated using specific agonists and antagonists. Cholinergic markers, choline acetyltransferase and acetylcholinesterase, differ quantitatively and qualitatively in retinas of different species. However, amacrine cells are cholinergic in all vertebrate species. Although they make up 1% of retinal neurons, they influence the activity of a majority of ganglion cells. Cholinergic effects in ganglia are mediated through nicotinic and muscarinic receptors. Both of these types of cholinergic receptors are heterogeneous. They have yet to be investigated for their subtypes using specific agonists and antagonists. Although the role of cholinergic retinal neurons in the processing of visual information is not known, their input to ganglion cells generally increases the rate of spontaneous activity or the number of action potentials in light-evoked responses. Thus, the cholinergic input seems to modify the overall neuronal input to the ganglion cells from the receptive fields. Endothelial cells of blood vessels contain muscarinic receptors, which are activated by ACh to cause relaxation. Although retinal blood vessels provide recognizable characteristic signs in diabetes mellitus and hypertensive disease, no information is available on the muscarinic receptors of these vessels.

Sastry, B. V. Rama

The Journal of Ocular Pharmacology **1**(2):201-226, 1985.

Other support: U. S. Public Health Service and the National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

KINETICS OF [^{3}H]MPP$^+$ UPTAKE IN DOPAMINERGIC NEURONS OF MOUSE: REGIONAL EFFECTS OF MPTP NEUROTOXICITY

In an effort to determine the specificity of MPTP/MPPP$^+$ toxicity with respect to the dopaminergic systems, the effect of prior MPTP treatment on [^{3}H]MPP$^+$ uptake in the striatum and olfactory tubercle of BALB/cBy mice was examined. Kinetic analysis of [^{3}H]MPP$^+$ uptake indicated a reduction of V_{max} values in both striatum (49%, $P<0.05$) and olfactory tubercle (26%, $P<0.05$). MPTP treatment did not significantly alter the K_m in either region, although MPP$^+$ accumulates in both olfactory tubercle and striatum, these dopaminergic systems show different sensitivity to the neurotoxicity of MPTP/MPP$^+$.

Sershen, H. et al.

European Journal of Pharmacology **126**:337-339, 1986.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, NY.

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

EFFECT OF AMPHETAMINE ON 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) NEUROTOXICITY IN MICE

Amphetamine has been shown to either potentiate or protect against MPTP neurotoxicity. The time course of changes in dopamine and its metabolites was examined after MPTP, amphetamine, or MPTP plus amphetamine administration. Results suggest that under conditions of granular depletion and release of dopamine by 10 mg/kg amphetamine, increased MPTP neurotoxicity occurs. Amphetamine injections at 2-5 mg/kg prevents the decline in dopamine possibly by blockade of the uptake of MPP^+, rather than by an inhibition of monoamine oxidase.

Sershen, H. et al.

Neuropharmacology **25**(8):927-930, 1986.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, NY.

EFFECT OF NICOTINE AND AMPHETAMINE ON THE NEUROTOXICITY OF *N*-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) IN MICE

The present results show the potentiating effect of amphetamine on the ability of MPTP to destroy dopaminergic neurons in striatum of the mouse. A single injection of MPTP (8 mg/kg, retro-orbital) reduced the binding of [^{3}H]mazindol, a marker for dopamine terminals, by 24%. When D-amphetamine (10 mg/kg, s.c.) was given 20 min prior to MPTP, the binding of [^{3}H]mazindol, measured 3-5 days later, was reduced by 58%. It is proposed that the mechanism of this potentiation primarily involves an increased release of dopamine by D-amphetamine, and free radical-mediated processes. Although nicotine also releases dopamine from the striatum, no effect was observed when it was administered prior to MPTP. The lack of effect is probably related to short duration of action of nicotine and the modest effect on release of dopamine as compared to that of amphetamine.

Sershen, H. et al.

Neuropharmacology **25**(11):1231-1234, 1986.

From the Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

SUPEROXIDE RADICAL-MEDIATED ALTERATION OF SYNAPTOSOME MEMBRANE STRUCTURE AND HIGH-AFFINITY γ-[^{14}C]AMINOBUTYRIC ACID UPTAKE

Mouse cortical synaptosomal structure and function are altered when exposed to hypoxanthine/xanthine oxidase (HPX/XOD)-generated active oxygen/free radical species. The structure of both the synaptic vesicle and plasma membrane systems are altered by HPX/XOD treatment. The alteration of synaptic vesicle structure is exhib-

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ited by a significant increase in the cumulative length of nonsynaptic vesicle membrane per nerve terminal. With respect to the nerve terminal plasma membrane, the length of the perimeter of the synaptosome is increased as the membrane pulls away from portions of the terminal in blebs. The functional lesion generated by HPX/XOD treatment results in a reduction in selective high-affinity γ-[^{14}C]aminobutyric acid (GABA) uptake. Kinetic analysis of the reduction in high-affinity uptake reveals that the V_{max} is significantly altered whereas the K_m is not. Preincubation with specific active oxygen/free radical scavengers indicates that the superoxide radical is directly involved. This radical, most probably in the protonated perhydroxyl form, initiates lipid peroxidative damage of the synaptosomal membrane systems. Low-affinity [^{14}C]GABA transport is unaltered by the HPX/XOD treatment. The apparent ineffectiveness of free radical exposure on low-affinity [^{14}C]GABA transport coupled with its effectiveness in reducing high-affinity transport supports the idea that two separate and different amino acid uptake systems exist in CNS tissue, with the high-affinity being more sensitive (lipid-dependent) and/or more energy-dependent (Na^+,K^+-ATPase) than the low-affinity system.

Debler, E. A., *Sershen, H.*, Lajtha, A. and Gennaro, J. F., Jr.

The Journal of Neurochemistry **47**(6):1804-1813, 1986.

From the Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward's Island; and Department of Biology, New York University, New York.

V. *Pharmacology and Biochemistry*

INHIBITION OF SERINE PROTEASES BY PEPTIDYL FLUOREMETHYL KETONES

We have synthesized peptidyl fluoromethyl ketones that are specific inhibitors of the serine proteases α-chymotrypsin and porcine pancreatic elastase. By analogy with the corresponding aldehydes it is assumed that the fluoromethyl ketones react with the γ-OH group of the active site serine to form a stable hemiacetal. ^{19}F NMR studies of the chymotrypsin-bound trifluoromethyl ketone inhibitors Ac-Leu-*ambo*-Phe-CF_3 and Ac-*ambo*-Phe-CF_3 clearly indicate that the carbonyl carbon is tetrahedral at the active site of the enzyme. The inhibitor is bound as either the stable hydrate or the hemiacetal, involving the active site serine. The effect of varying the number of amino acid residues in the peptidyl portion of the inhibitor and the number of fluorines in the fluoromethyl ketone moiety is examined. In the series of trifluoromethyl ketone elastase inhibitors, the lowering of K_i concomitant with the change from a dipeptide analogue to a tetrapeptide analogue (Ac-Pro-*ambo*-Ala-CF_3, $K_i = 3 \times 10^{-5}$ M; Ac-Ala-Ala-Pro-*ambo*-Ala-CF_3, $K_i = 0.34 \times 10^{-6}$ M) correlates well with the variation in V/K for hydrolysis of the corresponding amide substrates. This trend is indicative of the

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inhibitors acting as transition-state analogues. In addition to chain length, the number of fluorine substituents also effects the K_i. In the case of chymotrypsin, the K_i for Ac-Leu-*ambo*-Phe-CF_3 is 30-fold lower than that for Ac-Leu-*ambo*-Ala-CF_2H (0.88×10^{-7} M vs 25×10^{-7} M). With elastase this trend is not as profound. In all cases, however, the difluoro- and trifluoromethyl ketones are better inhibitors than the monofluoromethyl and nonfluorinated analogues. This improvement must be associated with both the degree of hydration of the fluoromethyl ketones and the significant effect that fluorine substitution has on lowering the first pK_a of the hemiacetal hydroxyl. The latter change would cause the more fluorinated inhibitor to be able to interact better with the anionic hole near the active site. Fluorine substitution also lowers the k_{off} values for the inhibitors. With elastase the trifluoromethyl ketone tetrapeptide has a k_{off} of 1.25×10^{-5} s^{-1}, while the corresponding difluoromethyl compound has a k_{off} of 0.007 s^{-1}. The monofluoromethyl ketone inhibitor of chymotrypsin, Ac-Leu-*ambo*-Phe-CFH_2, is a weak competitive inhibitor ($K_i = 200 \times 10^{-6}$ M). It also demonstrates time-dependent irreversible inhibition with a second-order rate constant of 1.7 M^{-1} s^{-1}. The irreversible inhibition is accompanied by covalent modification of a histidine residue and by fluoride ion release as detected by ^{19}F NMR spectroscopy.

Imperiali, B. and *Abeles, R. H.*

Biochemistry **25**(13):3760-3767, 1986.

Other support: National Institutes of Health.

From the Graduate Department of Biochemistry, Brandeis University, Waltham, MA.

A VERSATILE SYNTHESIS OF PEPTIDYL FLUOROMETHYL KETONES

Peptidyl fluoromethyl ketones constitute an important class of inhibitors of a number of serine proteases such as chymotrypsin and elastase. This communication presents a general method for the synthesis of these peptide analogs. The standard protocol described can be used for the synthesis of many analog compounds which can be tested against specific serine proteases by the selection of an appropriate nitroalkane and suitably protected peptide component.

Imperiali, B. and *Abeles, R.*

Tetrahedron Letters **27**(2):135-138, 1986.

Other support: National Institutes of Health.

From the Department of Biochemistry, Brandeis University, Waltham, MA.

BARREL ROTATION AND PROSTRATION BY VASOPRESSIN AND NICOTINE IN THE VESTIBULAR CEREBELLUM

The aim of this study was to determine whether the primary sites for the action of vasopressin and nicotine in producing barrel rotation and prostration in rats were located in the modular cerebellum, *i.e.*, lobule X. When arginine vasopressin was

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administered into either the fourth ventricles or directly into the nodular cerebellum via chronically implanted cannulae, the rats displayed intermittent barrel rotation and clonic convulsions. The administration of nicotine into the same areas resulted in prostration, atonia and, occasionally, clonic convulsions. A few days after the nodular cerebellum was lesioned with kainic acid, the motor distrubances resulting from either agent were virtually abolished. Histologic studies revealed that kainic acid had destroyed Purkinje and other large neurons, but had left the granular neurons relatively intact. The administration of procaine into either the fourth ventricles or nodular cerebellum blocked the behavioral responses of either vasopressin or nicotine given into the fourth ventricles. It was concluded that the nodular cerebellum is a primary site for the motor disturbances produced by vasopressin and nicotine.

Maiti, A., Salles, K. S., Grassi, S., and *Abood, L. G.*

Pharmacology, Biochemistry & Behavior **25**:583-588, 1986.

Other support: Department of Health and Human Services.

From the Center for Brain Research, University of Rochester Medical Center, Rochester, NY.

BEHAVIOR AND RECEPTOR CHANGES AFTER KAINATE LESIONING OF NODULAR CEREBELLUM

A study was undertaken on the effects of kainic acid lesioning on the nodulus of the rat cerebellum on behavior and various brain receptors in conscious, freely moving rats. The basis for the study was the observation that barrel rotation and other motor effects induced by intraventricular administration of vasopressin and nicotine could be elicited by their administration into the nodular area of the cerebellum. Histology revealed a marked destruction of Purkinje, stellate, and Golgi cells in the area surrounding the site of kainate administration, with little effect on the granular cells. Immediately after administering 4–12 ng of kainic acid into the nodular cerebellum, rats exhibited circling movements, barrel rotation, and clonic convulsions accompanied by stereotypic head movements, aggressiveness, and gnawing-biting; effects gradually diminishing over 3 days. Receptor binding studies 4–14 days after kainate lesioning revealed a marked increase in ^{3}H-nicotine and ^{3}H-QNB binding in the surrounding cerebellar region, caudate nucleus, and hypothalamus, with no change in ^{3}H-dihydromorphine binding. The findings are consistent with the hypothesis that nicotinic and muscarinic pathways in the vestibular cerebellum, along with its connection to nigrostriatal dopaminergic systems, are involved in the mediation of barrel rotation, ataxia, and other motor disturbances resulting from administration of vasopressin or nicotine intraventricularly.

Maiti, A., Salles, K. S., Grassi, S., and *Abood, L. G.*

Pharmacology, Biochemistry & Behavior **25**:589-594, 1986.

Other support: Department of Health and Human Services.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

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BINDING OF HIGH DENSITY LIPOPROTEIN TO CULTURED FIBROBLASTS AFTER CHEMICAL ALTERATION OF APOPROTEIN AMINO ACID RESIDUES

Cultured extrahepatic cells possess a specific high affinity binding site (receptor) for high density lipoprotein (HDL) that is induced by cholesterol delivery to cells. To characterize the binding recognition site(s) on HDL, the ability of HDL to interact with cultured human fibroblasts was assayed after chemical alteration of specific apoprotein amino acid residues. Reduction and alkylation, acetylation, and cyclohexanedione treatment of HDL$_3$ had little or no effect on its cellular binding. Treatment of HDL$_3$ with tetranitromethane (TNM), however, caused a large dose-dependent decrease in binding, with maximum inhibition at 3 mM. Amino acid analysis of the TNM-treated particles showed specific alteration of tyrosine residues, but sodium dodecyl sulfate-gel electrophoresis demonstrated apoprotein cross-linking coincident with decreased binding. These results suggest that modification of HDL tyrosine residues and/or cross-linking of HDL apoproteins alters the ligand site recognized by the HDL receptor. Gradient gel electrophoresis, molecular sieve chromatography, and electron microscopy showed only minor changes in size distribution and shape of HDL$_3$ particles after treatment with 3 mM TNM, but at higher TNM concentrations, coalescence and aggregation of particles was evident. Treatment of HDL$_3$ with 3 mM TNM affected neither its promotion of the low affinity (receptor-independent) cholesterol efflux from cells nor its ability to accept cholesterol from an albumin suspension, yet promotion of high affinity (receptor-dependent) cholesterol efflux from cells was abolished. The finding that TNM treatment of HDL$_3$ decreases both its receptor binding and its promotion of cholesterol efflux from cells without substantial alteration of its physical properties supports the hypothesis that the HDL receptor functions to facilitate cholesterol transport from cells.

Brinton, E. A., Oram, J. F., Chen, C.-H., *Albers, J. J.*, and Bierman, E. L.

The Journal of Biological Chemistry **261**(1):495-503, 1986.

Other support: National Institutes of Health, National Research Service Award Fellowship, and R. J. Reynolds Industries, Inc.

From the Division of Metabolism, Endocrinology and Nutrition, University of Washington, Seattle.

KINETIC STUDIES OF THE REDUCTION OF NEUTROPHIL CYTOCHROME *b*-558 BY DITHIONITE

The reduction with dithionite of neutrophil cytochrome *b*-558, implicated in superoxide generation by activated neutrophils, was investigated by a stopped-flow technique in non-ionic-detergent extracts of the membranes and in crude membrane particles. The dependence of the pseudo-first-order rate constants on the concentration of dithionite was consistent with a mechanism of reduction that involves the dithionite anion monomer $SO_2^{\cdot-}$ as the reactive species. The estimated second-order rate constant was $7.8 \times 10^6 M^{-1} \cdot s^{-1}$ for Lubrol PX-solubilized cytochrome *b*-558 and $5.1 \times 10^6 M^{-1} \cdot s^{-1}$ for the membrane-bound protein. The similarity of the kinetic constants suggests that solubilization did not introduce gross changes in the reactive site. Imidazole and *p*-chloromercuribenzoate, known as inhibitors of NADPH oxidase, did not affect signi-

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Source: https://www.industrydocuments.ucsf.edu/docs/xqxk0000

ficantly cytochrome *b*-558 reduction rates. The reaction rate of cytochrome *b*-558 with dithionite exhibited a near-zero activation energy. The first-order rate constant for reduction decreased with increasing ionic strength, indicating a positive effective charge on the reacting protein.

Aviram, I. and Sharabani, M.

Biochemical Journal **237**:567-572, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, University of Tel Aviv, Tel Aviv, Israel.

ACTIVATION-DEPENDENT REDISTRIBUTION OF CELLULAR COMPONENTS ALTERS SUSCEPTIBILITY OF HUMAN NEUTROPHILS TO CROSS-LINKING AGENTS

Contrary to resting cells, neutrophils stimulated with concanavalin A resist inhibition by bifunctional N-hydroxysuccinimide esters. Con A prestimulated, cross-linker-treated cells released superoxide upon restimulation with PMA but did not respond to chemotactic peptides. Although rates of PMA-elicited NADPH oxidase activity were lowered by the treatment, the activation parameters, namely lag times of the reaction, were not altered. The protection by Con A against blockade by cross-linkers developed concomitantly to the activation of NADPH oxidase and indicated redistribution of cross-linker-susceptible cellular components responsible for activation with PMA. The identity of these components is discussed.

Aviram, I. and Sharabani, M.

Inflammation **10**(3):233-242, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

A POSSIBLE ROLE FOR PROTEIN PHOSPHORYLATION IN THE ACTIVATION OF THE RESPIRATORY BURST IN HUMAN NEUTROPHILS

Two-dimensional gel electrophoresis was used to study protein phosphorylation in granules, membranes, and soluble fractions from human neutrophils that had been loaded with $^{32}P_i$. In resting cells, label was incorporated primarily into proteins of the membranes and the soluble supernatant; little appeared in the granules. Activation of ^{32}P-loaded neutrophils resulted in an increase in the ^{32}P content of a small number of membrane and soluble proteins without a change in the labeling of the granule fraction. the identity of the proteins affected by activation depended on the activating agent used; all of the activating agents, however, caused an increase in the labeling of a group of ~48-kDa proteins that appeared to be distributed between the membranes and the soluble supernatant.

To investigate the role of phosphorylation in the activation of the respiratory burst oxidase, the incorporation of ^{32}P into phosphoproteins was studied in neutrophils from

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patients with chronic granulomatous disease. When these cells were exposed to phorbol myristate acetate, one of the agents used for the activation of normal neutrophils, the 48-kDa proteins in the membranes and supernatants failed to take up additional ^{32}P. Phosphorylation patterns in normal neutrophils activated under nitrogen were similar to the patterns seen with cells activated in air, suggesting that the differences in phosphorylation between normal and chronic granulomatous disease neutrophils did not represent secondary effects of the oxidants produced by the normal cells, but reflected primary biochemical differences between the normal and the defective phagocytes. We postulate from these results that the uptake of phosphate by the 48-kDa protein group may be involved in the activation of the respiratory burst oxidase.

Hayakawa, T., Suzuki, K., Suzuki, S., and *Babior, B. M.*

The Journal of Biological Chemistry **261** (20):9109-9115, 1986.

Other support: U. S. Public Health Service.

From the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA.

THE RESPIRATORY BURST OXIDASE OF HUMAN NEUTROPHILS

A superoxide-forming oxidase from activated human neutrophil membranes was solubilized by two slightly different methods, then purified by "dye-affinity" chromatography. Kinetic studies of the purified preparations gave V_{max} values of 5–10 μmol of O_2^-/min/mg of protein, and K_m values for NADH and NADPH that were in reasonable agreement with values determined previously using particulate and crude solubilized preparations of the respiratory burst oxidase. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed prominent bands at 67, 48, and 32 kDa, together with some minor contaminants, whereas gel electrophoresis under non-denaturing conditions gave a single major band that when eluted and re-electrophoresed in the presence of sodium dodecyl sulfate showed bands at 67, 48, 32 kDa. We believe that all three bands represent oxidase components. The flavin content of the purified enzyme was 20.4 ± 2.0 S.E. pmol of FAD/μg of protein, whereas heme averaged 0.1 ± 0.02 pmol/μg and ubiquinone could not be detected. Assuming that the enzyme is composed of one 67-kDa subunit, one 48-kDa subunit, and one 32-kDa subunit (*i.e.*, that its molecular mass is ~150 kDa), it can be calculated to have a turnover number of 700–1500 min^{-1}, in agreement with a value reported previously for oxidase in a particulate O_2^--forming system (Cross, A. R., [illegible], J. J., and Jones, O. T. G. (1985) *Biochem. J.* 226, 881–884), and to contain the following quantities of redox carriers (mol/mol): FAD, 3.0; heme, 0.015; ubiquinone, <0.06. It remains to be determined whether this preparation represents the complete respiratory burst oxidase or is only the pyridine nucleotide dehydrogenating component of a more complex enzyme.

Glass, G. A. DeLisle, D. M., DeTogni, P., and *Babior, B. M.*

The Journal of Biological Chemistry **261** (28):13247-13251, 1986.

Other support: National Institutes of Health.

From the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA.

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DIETARY NICOTINE AND ITS SIGNIFICANCE IN STUDIES ON TOBACCO SMOKING

There is increasing interest in ingestion of nicotine by nonsmokers, and popular assumption is that inhalation of tobacco smoke is the sole source of this alkaloid in body fluids of nonsmokers. However, sources other than tobacco (particularly dietary) have been largely overlooked. In the present study, we measured nicotine levels in various solanaceae (tomato, pepper and eggplant) utilizing radioimmunoassay in order to understand the role of these food sources in the ingestion of nicotine. Our findings showed large quantities of nicotine in ripe fruit, and even greater amounts in unripe fruit.

Castro, A. and Monji, N.

Biochemical Archives **2**(2):91-97, 1986.

From the Department of Pathology, University of Miami School of Medicine, Miami, FL, and Genetics Systems Corp., Seattle.

IRON MEDIATES PARAQUAT TOXICITY IN *ESCHERICHIA COLI*

The role of iron ions in paraquat toxicity was studied in bacterial system. We show that addition of ferrous iron led to an enhancement of the bacterial killing, whereas addition of chelating agents, such as nitrilotriacetate and desferrioxamine, markedly reduced, up to a total abolishment, the toxic effects. The calculated rates of bacterial killing are proportional to both paraquat and iron concentrations, and conform to the rate equation $dN/dt = -k[\text{paraquat}][Fe^{2+}]$. The killing constant for iron, k, is 24-fold smaller than the corresponding value for copper.

Mannitol, an $OH^{\cdot}$ scavenger, has a partial protective effect: 15-35% at concentrations range of 1-50 mM, respectively. Histidine, on the other hand, provided a more efficient protection that may be due to a combination of various effects. Induction of endogenous superoxide dismutase and catalase provided partial protection (about 25%).

These findings, together with an earlier study on the role of copper in paraquat toxicity indicate that transition metals play a central catalytic role in the production of the deleterious effects of paraquat, probably by redox cycling and producing $OH^{\cdot}$ via the site-specific Fenton reaction.

[illegible]bi, P., Kohen, R., Katzhendler, J. and *Chevion, M.*

The Journal of Biological Chemistry **261**(27):12472-12476, 1986.

Other support: United States-Israel Binational Foundation.

From the Departments of Cellular Biochemistry and Pharmaceutical Chemistry, Hebrew University of Jerusalem, Israel.

HUMAN PRION PROTEIN cDNA: MOLECULAR CLONING, CHROMOSOMAL MAPPING, AND BIOLOGICAL IMPLICATIONS

A human complementary DNA whose protein product is considered to be the major component of scrapie-associated fibrils in Creutzfeldt-Jakob disease, kuru, and Gerstmann-Straussler syndrome has been identified and characterized. The extensive

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homology of this gene sequence to the hamster PrP 27- to 30-kilodalton prion protein complementary DNA clone, and its existence as a single copy in the human genome, leads to the conclusion that this is the human prion gene. This human prion gene has been mapped to human chromosome 20, negating a direct link between the prion protein and Down's syndrome or the amyloid of Alzheimer's disease.

Liao, Y.-C. J., Lebo, R. V., *Clawson, G. A.* and Smuckler, E. A.

Science **233**:364-367, 1986.

Other support: National Institutes of Health.

From the Department of Pathology, University of California, San Francisco.

p60$^{v\text{-}src}$ ACTIVITY DETECTED IN THE CHROMAFFIN GRANULE MEMBRANE

Using monoclonal antibodies specific for p60$^{v\text{-}src}$ we have detected high levels of this kinase in adrenal medullary chromaffin tissue and in highly purified chromaffin granule (secretory vesicle) membranes. An immune complex kinase assay was applied to fractions of adrenal medullary tissue resolved on sucrose density gradients. Thirty-seven per cent of the total tissue p60$^{v\text{-}src}$ activity was found in association with chromaffin granule or granule membrane markers. Localization of a significant fraction of total cellular p60$^{v\text{-}src}$ activity to this secretory vesicle membrane suggests that the kinase may function in the regulation of neurotransmitter release.

Parsons, S. J. and *Creutz, C. E.*

Biochemical and Biophysical Research Communications **134**(2):736-742, 1986.

Other support: National Institute of Health and University of Virginia Diabetes Research and Training Center.

From the Departments of Microbiology and Pharmacology and the Programs in Neuroscience and Cell and Molecular Biology, University of Virginia, Charlottesville.

PHOSPHORYLATION OF A CHROMAFFIN GRANULE-BINDING PROTEIN IN STIMULATED CHROMAFFIN CELLS

A procedure was devised to determine whether in the stimulated chromaffin cell phosphate is incorporated into specific proteins ("chromobindins") that bind to chromaffin granule membranes in a Ca^{2+}-dependent manner. Cells were preincubated with ^{32}P-labeled orthophosphate, then challenged with secretory stimuli. A postmicrosomal supernatant fraction was prepared from the cells and incubated with unlabeled chromaffin granule membranes in the presence of 5 mM Ca^{2+}. Proteins that bound to the membranes were isolated by centrifugation and examined for ^{32}P content by electrophoresis and autoradiography. Stimulation by carbamylcholine, nicotine, 56 mM K^+, or 2 mM Ba^{2+} led to the incorporation of ^{32}P into a 37-kDa protein that had previously been characterized as a substrate for protein kinase C *in vitro* chromobindin 9, or CB9. Incorporation of ^{32}P into this protein was dependent on extracellular Ca^{2+} and followed a time course that paralleled secretion of catecholamines, returning to base-line levels after 30 min, when secretion terminated. ^{32}P was also incorporated into a 58-kDa

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protein that may be tyrosine hydroxylase and into an unidentified 28-kDa protein in response to cell stimulation, but neither of these proteins bound to granule membranes in a Ca^{++}-dependent manner. Treatment of cells with phorbol 12,13-dibutyrate, an activator of protein kinase C, led to ^{32}P incorporation into the 37-kDa protein that was only 30% of the level obtained with nicotinic stimulation, suggesting that additional kinases may be involved in phosphorylating this protein in the stimulated cell.

Michener, M. L., Dawson, W. B., and *Creutz, C. E.*

The Journal of Biological Chemistry **261**(14):6548-6555, 1986.

Other support: National Institutes of Health, the National Science Foundation and the University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology and the Programs in Neuroscience and Cell and Molecular Biology, University of Virginia, Charlottesville.

WHEN DO METAL COMPLEXES PROTECT THE BIOLOGICAL SYSTEM FROM SUPEROXIDE TOXICITY AND WHEN DO THEY ENHANCE IT?

Many copper and iron complexes can be reduced by O_2^- as well as by H_2O_2. According to the rates of reduction and the concentration of O_2^- and H_2O_2, the metal complexes may serve either as catalyst of O_2^- dismutation or as catalysts of the reaction between O_2^- and H_2O_2 to form $OH^\cdot$ radical (Haber-Weiss reaction). Various factors which influence whether metal complexes protect the biological systems from superoxide toxicity or enhance it are discussed.

Czapski, G. and Goldstein, S.

Free Radical Research Communications **1**(3):157-161, 1986.

Other support: G. S. F. Neuherberg, West Germany.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

MECHANISM AND REACTION PRODUCTS OF THE OXIDATION OF Cu(I)-PHENANTHROLINE BY H_2O_2

We have suggested a possible reaction mechanism for the oxidation of the cuprous phenanthroline complex by H_2O_2 in the presence of formate and methanol. The cuprous phenanthroline complex was generated by pulse and γ radiolysis. We measured the decay kinetics of this complex as well as the chain length of this reaction. Our observations indicate that in this reaction $OH^\cdot$ is not formed directly, but through the decomposition of a metal-peroxo complex. This mechanism does not necessarily operate with other copper compounds, especially with copper complexes bound to a biological target.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **1**(5-6):373-380, 1985.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

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THE ROLE AND MECHANISM OF METAL IONS AND THEIR COMPLEXES IN ENHANCING DAMAGE IN BIOLOGICAL SYSTEMS OR IN PROTECTING THESE SYSTEMS FROM THE TOXICITY OF O_2^-

Copper complexes of 1,10-phenanthroline and some substituted 1,10-phenanthroline cleave DNA in the presence of a reducing agent and molecular oxygen. Generally, the damage is attributed to hydroxyl radicals which are formed through the Haber-Weiss reaction. It is assumed that this reaction occurs with the ternary metal complexes with the biological target and the mechanism is defined as the "site specific mechanism." In these systems, O_2^- drives the cycle through the reduction of copper(II). On the other hand, these same copper complexes catalyze the dismutation of O_2^- and thus should protect the systems from O_2^- toxicity. In this article, the toxicity of these complexes is explained on kinetic grounds. A general discussion on the various factors which could cause the metal ions or their complexes to act either as protectors from O_2^- toxicity or as sensitizers of toxic effects of O_2^- is given.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **2**:3-11, 1986.

Other support: Israel Academy of Science.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

MECHANISM AND REACTION PRODUCTS OF THE OXIDATION OF CU(I)-PHENANTHROLINE BY H_2O_2

We have suggested a possible reaction mechanism for the oxidation of the cuprous phenanthroline complex by H_2O_2 in the presence of formate and methanol. The cuprous phenanthroline complex was generated by pulse and γ radiolysis. We measured the decay kinetics of this complex as well as the chain length of this reaction. Our observations indicate that in this reaction OH· is not formed directly, but through the decomposition of a metal-peroxo complex. This mechanism does not necessarily operate with other copper compounds, especially with copper complexes bound to a biological target.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **1**:373-380, 1985.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

BACTERICIDAL EFFECT OF H_2O_2 AND DNA DAMAGE IN xthA MUTANTS OF *E. COLI*

Hydrogen peroxide is a normal metabolite in aerobic cells. Although not a very toxic species on its own, it may react with transition metals or metal chelates to produce

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radicals that have been shown to damage membrane lipids and nucleic acids. *E. coli* cells bearing functional DNA repair genes are resistant to millimolar concentrations of H_2O_2. Endonuclease III deficient (xthA) and recA mutants have been shown to be hypersensitive to H_2O_2. We have used these mutants to investigate the role of endogenous iron and copper in promoting the DNA damaging and bactericidal effects of H_2O_2 on growing bacterial cells.

The key role of transition metal ions in the toxicity of H_2O_2 is well documented. However, addition of various chelating agents, such as EDTA, DTPA and Desferrioxamine to growing xthA cells just before the introduction of H_2O_2 had only a slight protective effect. In contrast, preincubation of these cells with chelators, including OP, followed by washing and resuspension in growth medium had a marked protective effect. Results using cells grown in metal-poor medium showed that killing by H_2O_2 was proportional to the intrinsic iron content of the cells, whereas the killing of resting cells by copper-ascorbic acid was proportional to the amount of Cu^{++} absorbed to the cells.

Treatment of growing cultures of wild type or mutant cells with non-lethal concentrations of H_2O_2 caused an appreciable number of single strand breaks (SSB). With bactericidal concentrations we found a correlation between the killing effect of H_2O_2 or H_2O_2OP and the extent of DNA degradation (SSB and DSB). The hypersensitivity of xthA and recA cells to the bactericidal effect of H_2O_2 correlated with an increase in the number of SSB. Since recA cells are also hypersensitive to H_2O_2 and a great part of the SSB is repairable upon incubation in growth medium, it follows that the repair of the majority of the lesions does not involve exonuclease III which explains the almost normal sensitivity of xthA cells to γ-radiation.

Aronovitch, J., Samuni, A., Godinger, D., Greenbaum, M. and *Czapski, G.*

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B.V., 1986, pp. 343–345.

Other support: GSF Munich.

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SOD ACTIVITY *VERSUS* TOXICITY OF TERNARY COMPLEXES OF SOME COPPER COMPLEXES WITH DNA

It has been demonstrated that degradation of double-stranded DNA occurs in the presence of Cu(II) ions, 1,10-phenanthroline (OP), a reducing agent and O_2. The copper complex of 5-NO_2OP was found to be more effective than OP in cleaving DNA, while that of 2,2-bipyridyl (bipy) did not degrade DNA under the same conditions. The mechanism of the cleavage reaction was assumed to be through a site specific mechanism where the bound Cu(I) complex to DNA is oxidized by H_2O_2 to form the $OH^{\cdot}$ radical at the binding site. This mechanism explains the toxicity of the OH formed as it is formed at the binding site. Our observations indicate that no ligand dissociation takes place from bound CUL_2^{+} in the case of 5-NO_2OP but dissociation is very high in the case of bipy. In the latter case, the concentration of free CuL_2^{+} exceeds that of bound CUL_2^{+} and most of the oxidizing radicals are formed in the free solution. In the case of OP and

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5-NO_2OP, most of CuL_2^+ is bound to DNA and thus the majority of the $OH^\cdot$ is formed at the binding site in a site-specific mechanism.

Czapski, G. and Goldstein, S.

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B. V., 1986, pp. 64-66.

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SUPEROXIDE DISMUTASE ACTIVITY OF SOME COPPER PHENANTHROLINE COMPLEXES AND THE MECHANISM OF THE OXIDATION OF THE VARIOUS CUPROUS COMPLEXES BY H_2O_2

A large number of copper compounds have been tested for the rate at which they react with O_2^- and some of them were found able to catalyze its dismutation with an efficiency similar to that of Cu-Zn SOD. In view of the role of CU(II) in cleaving DNA in the presence of 1,10-phenanthroline (OP), a reducing agent, and O_2^- or H_2O_2, we have studied the kinetics and mechanism of the reactions of some copper complexes of OP and substituted OP with O_2^- and H_2O_2.

Our results lead us to conclude that during the oxidation of $(OP)_2CU^+$ by H_2O_2, initially no $OH^\cdot$ is formed, but either $(OP)_2CU^{3+}$ or $(OP)_2CuH_2O_2^{3+}$ are active intermediates. We cannot distinguish between these two species because their properties are unknown and their kinetic behavior is identical.

Goldstein, S. and *Czapski, G.*

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B. V., 1986, pp. 64-66.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

IN VIVO DEGRADATION OF BACTERIAL DNA BY H_2O_2 AND O-PHENANTHROLINE

1:10-Orthophenanthroline (OP) enhances killing of *E. coli* cells by H_2O_2 and the *in vivo* fragmentation of the bacterial DNA. There is a good correlation between the bactericidal effect and the number of single and double strand breaks produced in the bacterial DNA. The results suggest that intrinsic metal ions are involved in the bactericidal effect of OP and superoxide radical is probably not an obligatory intermediate.

Aronovitch, J., Samuni, A., Godinger, D., and *Czapski, G.*

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers, B. V., 1986, P. 346-348.

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From the School of Medicine and Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

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IMMUNOBLOTTING OF KERATIN POLYPEPTIDES EXTRACTED FROM TISSUES PRESERVED IN STANDARD HISTOLOGIC FIXATIVES

Cytoskeletal polypeptides from fresh placental tissue, tissue stored at −30°C, and tissue fixed in 10% buffered formalin, Bouin's solution, and Carnoy's solution were extracted, separated by electrophoresis, and immunoblotted using monoclonal antibodies immunoreactive with keratin polypeptides. Storage of the placental tissue at −30°C, or fixation in Carnoy's solution did not alter the extractability, migration pattern, or immunoreactivity of the keratin polypeptides. Keratin polypeptides could not be adequately demonstrated in extracts prepared from formalin- or Bouin's solution-fixed tissues. Several unmasking procedures used on tissues before extraction and on nitrocellulose blots before application of primary antibodies failed to unmask keratin polypeptides, either in Coomassie blue-stained gels or in immunoblots reacted with anti-keratin antibodies. These data indicate that Carnoy's solution is the fixative of choice for tissues in which electrophoretic and immunoblotting analyses of keratin polypeptides might be required.

Clark, R. K., and *Damjanov, I.*

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From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

SUPPRESSION OF NONSPECIFIC BINDING OF AVIDIN-BIOTIN COMPLEX (ABC) TO PROTEINS ELECTROBLOTTED TO NITROCELLULOSE PAPER

Nitrocellulose blots of cell extracts reacted in sequence with biotinylated lectins and horseradish peroxidase-labeled avidin-biotin complex (ABC) often show considerable nonspecific staining of protein bands. Experiments were performed to determine which of the components of the ABC were responsible for this and whether or not the nature and ionic strength of the buffer used could alter this binding. Furthermore, as powdered non-fat milk has been proposed as a possible blocking agent for nonspecific binding of ABC, we sought to determine if it would adequately block that binding in our system. The initial experiments showed that nonspecific binding of ABC to proteins transferred to nitrocellulose membranes was due to the avidin component of the ABC; little, if any, binding was seen if biotin alone was incubated with these blots. The spurious binding was shown to be primarily due to the high affinity of avidin to proteins electroblotted to nitrocellulose, when incubated in low-salt buffers. Low-fat milk added to the buffer reduced overall nonspecific reactivity but produced additional artifacts in the form of bands that were not seen in other preparations. Nonspecific avidin binding to proteins transferred to nitrocellulose can therefore be effectively reduced by adding extra salt to buffers, whereas the addition of nonfat dry milk does not seem suitable for this purpose.

Clark, R. K., Tani, Y., and *Damjanov, I.*

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Other support: National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

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